

**WHAT IS CLAIMED IS:**

1. An isolated nucleic acid comprising a strand that is a template for an RNA molecule that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, and wherein said sense and antisense nucleic acid sequences form double-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence.
2. The isolated nucleic acid of claim 1, wherein said antisense nucleic acid sequence is complementary to a viral mRNA sequence.
3. The isolated nucleic acid of claim 1, wherein said antisense nucleic acid sequence is complementary to a mammalian mRNA sequence.
4. The isolated nucleic acid of claim 1, wherein said sense nucleic acid sequence is at least 15 nucleotides in length.
5. The isolated nucleic acid of claim 1, wherein said sense nucleic acid sequence is from about 15 to about 300 nucleotides in length.
6. The isolated nucleic acid of claim 1, wherein said sense nucleic acid sequence is from about 15 to about 50 nucleotides in length.
7. The isolated nucleic acid of claim 1, wherein said sense nucleic acid sequence comprises the sequence as set forth in SEQ ID NO: 1, 2, 3, 4, 5, 6, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, or 54.
8. The isolated nucleic acid of claim 1, wherein said cis-acting ribozyme sequence is 3' of said sense nucleic acid sequence or said antisense nucleic acid sequence.

9. The isolated nucleic acid of claim 1, wherein said cis-acting ribozyme sequence is 3' of said sense nucleic acid sequence and said antisense nucleic acid sequence.

5 10. The isolated nucleic acid of claim 1, wherein said cis-acting ribozyme sequence is 5' of said sense nucleic acid sequence or said antisense nucleic acid sequence.

11. The isolated nucleic acid of claim 1, wherein said cis-acting ribozyme sequence is 5' of said sense nucleic acid sequence and said antisense nucleic acid sequence.

10 12. The isolated nucleic acid of claim 1, wherein said cis-acting ribozyme sequence is between said sense nucleic acid sequence and said antisense nucleic acid sequence.

13. The isolated nucleic acid of claim 1, wherein said nucleic acid is double stranded.

15 14. The isolated nucleic acid of claim 1, wherein said nucleic acid is single stranded.

15. The isolated nucleic acid of claim 1, wherein said nucleic acid is DNA.

16. The isolated nucleic acid of claim 1, wherein said nucleic acid is RNA.

20 17. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a restriction site.

18. The isolated nucleic acid of claim 1, wherein said nucleic acid is a plasmid.

25 19. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a promoter sequence that promotes transcription of said RNA molecule.

30 20. The isolated nucleic acid of claim 19, wherein said promoter sequence is a tissue-specific promoter, cell-specific promoter, or pathogen-specific promoter.

21. The isolated nucleic acid of claim 19, wherein said promoter sequence promotes transcription by RNA polymerase II.

5 22. The isolated nucleic acid of claim 19, wherein said promoter sequence promotes transcription by RNA polymerase III.

23. The isolated nucleic acid of claim 19, wherein said promoter sequence is an H1 promoter sequence or a U6 promoter sequence.

10 24. The isolated nucleic acid of claim 19, wherein said promoter sequence is selected from the group consisting of a mouse albumin enhancer promoter sequence, a transferrin promoter sequence, a probasin promoter sequence, and a whey acidic protein promoter sequence.

15 25. The isolated nucleic acid of claim 19, wherein said promoter sequence is selected from the group consisting of a keratin 7 promoter sequence, a keratin 13 promoter sequence, and a keratin enhancer promoter sequence.

20 26. The isolated nucleic acid of claim 1, wherein said RNA molecule is transcribed from said nucleic acid when said nucleic acid is within a cell.

27. The isolated nucleic acid of claim 26, wherein said cell is selected from the group consisting of kidney cells, skin cells, liver cells, neurons, muscle cells, and lymphocytes.

25 28. The isolated nucleic acid of claim 1, wherein said strand is a template for more than one cis-acting ribozyme sequence.

29. The isolated nucleic acid of claim 28, wherein each of said more than one cis-acting ribozyme sequence is different.

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30. The isolated nucleic acid of claim 28, wherein one of said more than one cis-acting ribozyme sequence is 5' of said sense nucleic acid sequence and said antisense nucleic acid sequence.

5 31. The isolated nucleic acid of claim 30, wherein another of said more than one cis-acting ribozyme sequence is 3' of said sense nucleic acid sequence and said antisense nucleic acid sequence.

10 32. The isolated nucleic acid of claim 28, wherein said sense nucleic acid sequence and said antisense nucleic acid sequence are each flanked by at least one of said more than one cis-acting ribozyme sequence.

33. The isolated nucleic acid of claim 32, wherein said strand is a template for three cis-acting ribozyme sequences.

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34. An isolated nucleic acid comprising a strand that is a template for an RNA molecule that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense and  
20 antisense nucleic acid sequences form a single-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence, and wherein said single-stranded RNA contains no more than one hairpin loop structure and lacks RNA cleaving activity.

25 35. The isolated nucleic acid of claim 34, wherein said cis-acting ribozyme sequence is 3' of said sense nucleic acid sequence and said antisense nucleic acid sequence.

36. The isolated nucleic acid of claim 34, wherein said cis-acting ribozyme sequence is 5' of said sense nucleic acid sequence and said antisense nucleic acid sequence.

30 37. The isolated nucleic acid of claim 34, wherein said nucleic acid is double stranded.

38. The isolated nucleic acid of claim 34, wherein said nucleic acid is single stranded.

39. The isolated nucleic acid of claim 34, wherein said nucleic acid is DNA.

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40. The isolated nucleic acid of claim 34, wherein said nucleic acid is RNA.

41. The isolated nucleic acid of claim 34, wherein said nucleic acid comprises a promoter sequence that promotes transcription of said RNA molecule.

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42. The isolated nucleic acid of claim 34, wherein said promoter sequence promotes transcription by RNA polymerase II.

43. The isolated nucleic acid of claim 34, wherein said strand is a template for more than one cis-acting ribozyme sequence.

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44. The isolated nucleic acid of claim 43, wherein each of said more than one cis-acting ribozyme sequence is different.

45. The isolated nucleic acid of claim 43, wherein one of said more than one cis-acting ribozyme sequence is 5' of said sense nucleic acid sequence and said antisense nucleic acid sequence, and wherein another of said more than one cis-acting ribozyme sequence is 3' of said sense nucleic acid sequence and said antisense nucleic acid sequence.

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46. An isolated nucleic acid comprising an RNA strand that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, and wherein said sense and antisense nucleic acid sequences form double-stranded RNA upon cleavage of said RNA strand by said cis-acting ribozyme sequence.

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47. The isolated nucleic acid of claim 46, wherein said nucleic acid is single stranded.

48. The isolated nucleic acid of claim 46, wherein said RNA strand comprises more than one cis-acting ribozyme sequence.

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49. The isolated nucleic acid of claim 48, wherein each of said more than one cis-acting ribozyme sequence is different.

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50. The isolated nucleic acid of claim 48, wherein said sense nucleic acid sequence and said antisense nucleic acid sequence are each flanked by at least one of said more than one cis-acting ribozyme sequence.

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51. An isolated nucleic acid comprising an RNA strand that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense and antisense nucleic acid sequences form a single-stranded RNA upon cleavage of said RNA strand by said cis-acting ribozyme sequence, and wherein said single-stranded RNA contains no more than one hairpin loop structure and lacks RNA cleaving activity.

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52. The isolated nucleic acid of claim 51, wherein said nucleic acid is single stranded.

53. The isolated nucleic acid of claim 51, wherein said RNA strand comprises more than one cis-acting ribozyme sequence.

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54. The isolated nucleic acid of claim 53, wherein each of said more than one cis-acting ribozyme sequence is different.

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55. The isolated nucleic acid of claim 53, wherein one of said more than one cis-acting ribozyme sequence is 5' of said sense nucleic acid sequence and said antisense nucleic acid sequence, and wherein another of said more than one cis-acting ribozyme

sequence is 3' of said sense nucleic acid sequence and said antisense nucleic acid sequence.

56. A composition comprising a pharmaceutically acceptable carrier and an isolated nucleic acid selected from the group consisting of:

(a) isolated nucleic acids comprising a strand that is a template for an RNA molecule that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, and wherein said sense and antisense nucleic acid sequences form double-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence,

(b) isolated nucleic acids comprising a strand that is a template for an RNA molecule that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense and antisense nucleic acid sequences form a single-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence, and wherein said single-stranded RNA contains no more than one hairpin loop structure and lacks RNA cleaving activity,

(c) isolated nucleic acids comprising an RNA strand that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, and wherein said sense and antisense nucleic acid sequences form double-stranded RNA upon cleavage of said RNA strand by said cis-acting ribozyme sequence, and

(d) isolated nucleic acids comprising an RNA strand that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense and antisense nucleic acid sequences form a single-stranded RNA upon cleavage of said RNA strand by said cis-acting ribozyme sequence, and wherein said single-stranded RNA contains no more than one hairpin loop structure and lacks RNA cleaving activity.

57. An isolated cell comprising an isolated nucleic acid selected from the group consisting of:

(a) isolated nucleic acids comprising a strand that is a template for an RNA molecule that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, and wherein said sense and antisense nucleic acid sequences form double-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence,

(b) isolated nucleic acids comprising a strand that is a template for an RNA molecule that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense and antisense nucleic acid sequences form a single-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence, and wherein said single-stranded RNA contains no more than one hairpin loop structure and lacks RNA cleaving activity,

(c) isolated nucleic acids comprising an RNA strand that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, and wherein said sense and antisense nucleic acid sequences form double-stranded RNA upon cleavage of said RNA strand by said cis-acting ribozyme sequence, and

(d) isolated nucleic acids comprising an RNA strand that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense and antisense nucleic acid sequences form a single-stranded RNA upon cleavage of said RNA strand by said cis-acting ribozyme sequence, and wherein said single-stranded RNA contains no more than one hairpin loop structure and lacks RNA cleaving activity.

58. A virus comprising an isolated nucleic acid selected from the group consisting of:



(a) isolated nucleic acids comprising a strand that is a template for an RNA molecule that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, and wherein said sense and antisense nucleic acid sequences form double-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence,

(b) isolated nucleic acids comprising a strand that is a template for an RNA molecule that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense and antisense nucleic acid sequences form a single-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence, and wherein said single-stranded RNA contains no more than one hairpin loop structure and lacks RNA cleaving activity,

(c) isolated nucleic acids comprising an RNA strand that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, and wherein said sense and antisense nucleic acid sequences form double-stranded RNA upon cleavage of said RNA strand by said cis-acting ribozyme sequence, and

(d) isolated nucleic acids comprising an RNA strand that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense and antisense nucleic acid sequences form a single-stranded RNA upon cleavage of said RNA strand by said cis-acting ribozyme sequence, and wherein said single-stranded RNA contains no more than one hairpin loop structure and lacks RNA cleaving activity.

59. The virus of claim 58, wherein said virus is a retrovirus, adenovirus, herpes virus, adeno-associated virus, lentivirus, baculovirus, cauliflower mosaic virus, tobacco mosaic virus, togavirus, poliovirus, cytomegalovirus, Paramyxovirus, Epstein-Barr virus, human papillomavirus, or hepatitis C virus.

60. A nonhuman transgenic animal, wherein the genome of said nonhuman transgenic animal comprises a nucleic acid sequence, present on one strand, that is a template for an RNA molecule that comprises:

(a) a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, and wherein said sense and antisense nucleic acid sequences form double-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence, or

(b) a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense and antisense nucleic acid sequences form a single-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence, and wherein said single-stranded RNA contains no more than one hairpin loop structure and lacks RNA cleaving activity.

61. The nonhuman transgenic animal of claim 60, wherein said nonhuman transgenic animal is a mouse.

62. A method of identifying sequences capable of inducing RNA interference against a target mRNA, said method comprising:

(a) introducing a vector preparation into cells, wherein each vector of said vector preparation comprises:

- (1) a target nucleic acid sequence, wherein said target nucleic acid sequence is a template for said target mRNA;
- (2) a reporter nucleic acid sequence, wherein said reporter nucleic acid sequence encodes a polypeptide, and wherein said target nucleic acid sequence and said reporter nucleic acid sequence are transcribed as a single fusion mRNA; and

(3) a promoter sequence region, wherein said promoter sequence region comprises: (i) a member of a plurality of test nucleic acid sequences, and (ii) two promoter sequences operably linked to said member in an arrangement that promotes transcription of both strands of said member;

5 (b) identifying at least one cell lacking said polypeptide; and

(c) obtaining the sequence of said member from said cell identified in step (b), thereby identifying said sequence as being capable of inducing RNA interference against said target mRNA.

10 63. The method of claim 62, wherein said polypeptide is a fluorescent polypeptide.

64. The method of claim 62, wherein said polypeptide is lethal to said cell.

15 65. A method for reducing the level of an mRNA in a cell, said method comprising introducing an isolated nucleic acid into said cell, wherein said isolated nucleic acid is selected from the group consisting of:

(a) isolated nucleic acids comprising a strand that is a template for an RNA molecule that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, and wherein said sense and antisense nucleic acid sequences form double-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence,

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(b) isolated nucleic acids comprising a strand that is a template for an RNA molecule that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense and antisense nucleic acid sequences form a single-stranded RNA upon cleavage of said RNA molecule by said cis-acting ribozyme sequence, and wherein said single-stranded RNA contains no more than one hairpin loop structure and lacks RNA cleaving activity,

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(c) isolated nucleic acids comprising an RNA strand that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, and wherein said sense and antisense nucleic acid sequences form double-stranded RNA upon cleavage of said RNA strand by said cis-acting ribozyme sequence, and

(d) isolated nucleic acids comprising an RNA strand that comprises a sense nucleic acid sequence, an antisense nucleic acid sequence, and a cis-acting ribozyme sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense and antisense nucleic acid sequences form a single-stranded RNA upon cleavage of said RNA strand by said cis-acting ribozyme sequence, and wherein said single-stranded RNA contains no more than one hairpin loop structure and lacks RNA cleaving activity, wherein the presence of said isolated nucleic acid within said cell reduces the level of said mRNA in said cell.

66. A mixture comprising at least two isolated nucleic acids, wherein one of said at least two isolated nucleic acids comprises a strand that is a template for an RNA molecule comprising a sense nucleic acid sequence and a first cis-acting ribozyme sequence, wherein another of said at least two isolated nucleic acids comprises a strand that is a template for an RNA molecule comprising an antisense nucleic acid sequence and a second cis-acting ribozyme sequence, and wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence.

67. An isolated nucleic acid comprising:

(a) a target nucleic acid sequence, wherein said target nucleic acid sequence is a template for target mRNA, and

(b) a promoter sequence region, wherein said promoter sequence region comprises (i) a nucleic acid sequence and (ii) two promoter sequences operably linked to said nucleic acid sequence in an arrangement that promotes transcription of both strands of said nucleic acid sequence, wherein said nucleic acid sequence comprises a sequence

present in said target nucleic acid sequence, and wherein transcription, within a cell, of said target nucleic acid sequence and both strands of said nucleic acid sequence is capable of inducing RNA interference against said target mRNA.

5      68.      The isolated nucleic acid of claim 67, wherein said target nucleic acid sequence is a viral sequence.

69.      The isolated nucleic acid of claim 67, wherein said target nucleic acid sequence is an HPV sequence.

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70.      The isolated nucleic acid of claim 67, wherein said target nucleic acid sequence is an HBV sequence.

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71.      The isolated nucleic acid of claim 67, wherein one of said two promoter sequences is a U6 promoter sequence.

72.      The isolated nucleic acid of claim 67, wherein one of said two promoter sequences is an H1 promoter sequence.

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73.      The isolated nucleic acid of claim 67, wherein said two promoter sequences are the same.

74.      The isolated nucleic acid of claim 67, wherein said two promoter sequences are different.

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75.      The isolated nucleic acid of claim 67, wherein said two promoter sequences are separated by no more than 200 base pairs.

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76.      The isolated nucleic acid of claim 67, wherein said two promoter sequences are separated by no more than 100 base pairs.

77. The isolated nucleic acid of claim 67, wherein said two promoter sequences are separated by no more than 50 base pairs.

78. The isolated nucleic acid of claim 67, wherein said promoter sequence region  
5 comprises more than two promoter sequences.

79. The isolated nucleic acid of claim 67, wherein said sequence present in said target nucleic acid sequence is between 15 and 50 nucleotides in length.

10 80. The isolated nucleic acid of claim 67, wherein said sequence present in said target nucleic acid sequence is between 18 and 25 nucleotides in length.

81. The isolated nucleic acid of claim 67, wherein said isolated nucleic acid comprises a reporter nucleic acid sequence, wherein said reporter nucleic acid sequence encodes a  
15 polypeptide, and wherein said target nucleic acid sequence and said reporter nucleic acid sequence are transcribed as a single fusion mRNA.

82. The isolated nucleic acid of claim 81, wherein said polypeptide is a fluorescent polypeptide.  
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83. The isolated nucleic acid of claim 81, wherein said polypeptide is a green fluorescent polypeptide.

84. A nucleic acid library comprising isolated nucleic acids, wherein each isolated  
25 nucleic acid comprises:

(a) a target nucleic acid sequence, wherein said target nucleic acid sequence is a template for target mRNA, and

(b) a promoter sequence region, wherein said promoter sequence region comprises (i) a nucleic acid sequence and (ii) two promoter sequences operably linked to said nucleic  
30 acid sequence in an arrangement that promotes transcription of both strands of said nucleic acid sequence, wherein said nucleic acid sequence is different for each isolated

nucleic acid, wherein said nucleic acid sequence of at least one of said isolated nucleic acids comprises a sequence present in said target nucleic acid sequence, and wherein transcription, within a cell, of said target nucleic acid sequence and both strands of said nucleic acid sequence of at least one of said isolated nucleic acids is capable of inducing  
5 RNA interference against said target mRNA.

85. The nucleic acid library of claim 84, wherein said target nucleic acid sequence is a viral sequence.

10 86. The nucleic acid library of claim 84, wherein said target nucleic acid sequence is an HPV sequence.

87. The nucleic acid library of claim 84, wherein said target nucleic acid sequence is an HBV sequence.

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88. The nucleic acid library of claim 84, wherein one of said two promoter sequences is a U6 promoter sequence.

89. The nucleic acid library of claim 84, wherein one of said two promoter sequences  
20 is an H1 promoter sequence.

90. The nucleic acid library of claim 84, wherein said two promoter sequences are the same.

25 91. The nucleic acid library of claim 84, wherein said two promoter sequences are different.

92. The nucleic acid library of claim 84, wherein said two promoter sequences are separated by no more than 200 base pairs.

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93. The nucleic acid library of claim 84, wherein said two promoter sequences are separated by no more than 100 base pairs.

94. The nucleic acid library of claim 84, wherein said two promoter sequences are separated by no more than 50 base pairs.

95. The nucleic acid library of claim 84, wherein said promoter sequence region comprises more than two promoter sequences.

96. The nucleic acid library of claim 84, wherein said sequence present in said target nucleic acid sequence is between 15 and 50 nucleotides in length.

97. The nucleic acid library of claim 84, wherein said sequence present in said target nucleic acid sequence is between 18 and 25 nucleotides in length.

98. The nucleic acid library of claim 84, wherein said isolated nucleic acid comprises a reporter nucleic acid sequence, wherein said reporter nucleic acid sequence encodes a polypeptide, and wherein said target nucleic acid sequence and said reporter nucleic acid sequence are transcribed as a single fusion mRNA.

99. The nucleic acid library of claim 84, wherein said polypeptide is a fluorescent polypeptide.

100. The nucleic acid library of claim 84, wherein said polypeptide is a green fluorescent polypeptide.

101. An isolated nucleic acid comprising:

(a) a target nucleic acid sequence, wherein said target nucleic acid sequence is a template for target mRNA, and

(b) a promoter sequence region, wherein said promoter sequence region comprises a promoter sequence operably linked to a nucleic acid sequence, wherein one



strand of said nucleic acid sequence is a template for a sense nucleic acid sequence and an antisense nucleic acid sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense nucleic acid sequence comprises a sequence present in said target mRNA, and wherein transcription,  
5 within a cell, of said target nucleic acid sequence and at least one strand of said nucleic acid sequence is capable of inducing RNA interference against said target mRNA.

102. The isolated nucleic acid of claim 101, wherein said target nucleic acid sequence is a viral sequence.

103. The isolated nucleic acid of claim 101, wherein said target nucleic acid sequence is an HPV sequence.

104. The isolated nucleic acid of claim 101, wherein said target nucleic acid sequence is an HBV sequence.

105. The isolated nucleic acid of claim 101, wherein said promoter sequence is a U6 promoter sequence or an H1 promoter sequence.

106. The isolated nucleic acid of claim 101, wherein said promoter sequence region comprises two promoter sequences operably linked to said nucleic acid sequence in an arrangement that promotes transcription of both strands of said nucleic acid sequence.

107. The isolated nucleic acid of claim 106, wherein said two promoter sequences are the same.

108. The isolated nucleic acid of claim 106, wherein said two promoter sequences are different.

109. The isolated nucleic acid of claim 106, wherein said two promoter sequences are separated by no more than 200 base pairs.

110. The isolated nucleic acid of claim 106, wherein said two promoter sequences are separated by no more than 100 base pairs.

5 111. The isolated nucleic acid of claim 106, wherein said two promoter sequences are separated by no more than 50 base pairs.

112. The isolated nucleic acid of claim 106, wherein said promoter sequence region comprises more than two promoter sequences.

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113. The isolated nucleic acid of claim 101, wherein said sequence present in said target nucleic acid sequence is between 15 and 50 nucleotides in length.

114. The isolated nucleic acid of claim 101, wherein said sequence present in said  
15 target nucleic acid sequence is between 18 and 25 nucleotides in length.

115. The isolated nucleic acid of claim 101, wherein the transcription product from at least one strand of said nucleic acid sequence is capable of forming a hairpin loop structure.

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116. The isolated nucleic acid of claim 115, wherein at least a portion of the stem portion of said hairpin loop structure is formed by said sense nucleic acid sequence and said antisense nucleic acid sequence.

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117. The isolated nucleic acid of claim 101, wherein said isolated nucleic acid comprises a reporter nucleic acid sequence, wherein said reporter nucleic acid sequence encodes a polypeptide, and wherein said target nucleic acid sequence and said reporter nucleic acid sequence are transcribed as a single fusion mRNA.

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118. The isolated nucleic acid of claim 117, wherein said polypeptide is a fluorescent polypeptide.

119. The isolated nucleic acid of claim 117, wherein said polypeptide is a green fluorescent polypeptide.

5 120. A nucleic acid library comprising isolated nucleic acids, wherein each isolated nucleic acid comprises a promoter sequence operably linked to a nucleic acid sequence, wherein one strand of said nucleic acid sequence is a template for a sense nucleic acid sequence and an antisense nucleic acid sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense  
10 nucleic acid sequence is different for each isolated nucleic acid, and wherein transcription, within a cell, of a target nucleic acid sequence and at least one strand of said nucleic acid sequence of at least one of said isolated nucleic acids is capable of inducing RNA interference against a target mRNA, said target nucleic acid sequence being a template for said target mRNA.

15 121. The nucleic acid library of claim 120, wherein each isolated nucleic acid comprises said target nucleic acid sequence.

20 122. The nucleic acid library of claim 120, wherein said sense nucleic acid sequence of at least one of said isolated nucleic acids comprises a sequence present in said target mRNA.

25 123. The nucleic acid library of claim 120, wherein said target nucleic acid sequence is a viral sequence.

124. The nucleic acid library of claim 120, wherein said target nucleic acid sequence is an HPV sequence.

30 125. The nucleic acid library of claim 120, wherein said target nucleic acid sequence is an HBV sequence.

126. The nucleic acid library of claim 120, wherein said promoter sequence is a U6 promoter sequence or an H1 promoter sequence.

127. The nucleic acid library of claim 120, wherein said isolated nucleic acids  
5 comprise two promoter sequences operably linked to said nucleic acid sequence in an arrangement that promotes transcription of both strands of said nucleic acid sequence.

128. The nucleic acid library of claim 127, wherein said two promoter sequences are the same.

10 129. The nucleic acid library of claim 127, wherein said two promoter sequences are different.

130. The nucleic acid library of claim 127, wherein said two promoter sequences are  
15 separated by no more than 200 base pairs.

131. The nucleic acid library of claim 127, wherein said two promoter sequences are separated by no more than 100 base pairs.

20 132. The nucleic acid library of claim 127, wherein said two promoter sequences are separated by no more than 50 base pairs.

133. The nucleic acid library of claim 127, wherein said isolated nucleic acids  
25 comprise more than two promoter sequences.

134. The nucleic acid library of claim 120, wherein said sequence present in said target  
nucleic acid sequence is between 15 and 50 nucleotides in length.

135. The nucleic acid library of claim 120, wherein said sequence present in said target  
30 nucleic acid sequence is between 18 and 25 nucleotides in length.

136. The nucleic acid library of claim 120, wherein said isolated nucleic acids comprise a reporter nucleic acid sequence, wherein said reporter nucleic acid sequence encodes a polypeptide, and wherein said target nucleic acid sequence and said reporter nucleic acid sequence are transcribed as a single fusion mRNA.

137. The nucleic acid library of claim 136, wherein said polypeptide is a fluorescent polypeptide.

138. The nucleic acid library of claim 136, wherein said polypeptide is a green fluorescent polypeptide.

139. A method for making a library comprising isolated nucleic acids, wherein each isolated nucleic acid comprises a nucleic acid sequence, wherein one strand of said nucleic acid sequence is a template for a sense nucleic acid sequence and an antisense nucleic acid sequence, wherein said sense nucleic acid sequence is complementary to said antisense nucleic acid sequence, wherein said sense nucleic acid sequence is different for each isolated nucleic acid, wherein transcription, within a cell, of a target nucleic acid sequence and at least one strand of said nucleic acid sequence of at least one of said isolated nucleic acids is capable of inducing RNA interference against a target mRNA, and wherein said target nucleic acid sequence is a template for said target mRNA, said method comprising:

(a) obtaining a nucleic acid collection comprising nucleic acid molecules, wherein one strand of each nucleic acid molecule comprises said sense nucleic acid sequence or said antisense nucleic acid sequence, wherein said sense nucleic acid sequence or said antisense nucleic acid sequence is different for each nucleic acid molecule, wherein said one strand of each nucleic acid molecule comprises a first sequence and a second sequence, wherein said first sequence is complementary to said second sequence, and wherein said first and second sequences are located 3' of said sense nucleic acid sequence or said antisense nucleic acid sequence of each nucleic acid molecule, and

(b) amplifying said nucleic acid collection in an amplification reaction under conditions wherein the 3' end of each nucleic acid molecule is extended using a portion of the 5' end of each nucleic acid molecule as a template to form an extended nucleic acid collection comprising extended nucleic acid molecules, wherein said amplification  
5 reaction amplifies said extended nucleic acid molecules, wherein one strand of each extended nucleic acid molecule comprises said sense nucleic acid sequence and said antisense nucleic acid sequence, and wherein said extended nucleic acid collection is said library.

10 140. The method of claim 139, wherein said method comprises removing a portion of the sequence located between said sense nucleic acid sequence and said antisense nucleic acid sequence of each extended nucleic acid molecule.

15 141. The method of claim 140, wherein, after said removing step, said sense nucleic acid sequence and said antisense nucleic acid sequence of each extended nucleic acid molecule is separated by 4 to 20 nucleotides.

142. The method of claim 139, wherein said method comprises inserting each extended nucleic acid molecule into an expression vector.

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